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Pulmonary Function in Normal and Elastase-Treated Hamsters Exposed to a Complex Mixture of Olefin-Ozone-Sulfur Dioxide Products

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16. ABSTRACT

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JOURNAL ARTICLE

Pulmonary Function in Normal and Elastase-Treated Hamsters Exposed to a Complex Mixture of Olefin–Ozone–Sulfur Dioxide Reaction Products[1,2]

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An elastase-induced emphysema model was utilized to determine if hamsters with preexisting lung disease were more susceptible to lung damage from air pollutant exposure. Male golden hamsters, divided into two treatment groups, were given a single intratracheal injection of either 6 units of porcine pancreatic elastase (EMP) or buffer (CNT). After a 4-week recovery period, equal numbers of each group were exposed 23 hr/day × 28 day to filtered air (AIR) or to the complex by-products from a dark phase reaction mixture of trans-2-butene, ozone, and sulfur dioxide (MIX). Lung function measurements on the elastase-treated groups showed changes consistent with mild emphysema. There were no significant differences in lung volumes or lung compliance between the AIR- and MIX-exposed animals. However, the nitrogen washout slope decreased ($P < 0.05$), and the diffusing capacity for carbon monoxide increased ($P < 0.05$) in both the CNT and EMP hamsters exposed to the MIX. The change in diffusing capacity was greater ($P < 0.05$) in normal hamsters than in hamsters with emphysema, and it is hypothesized that animals with impaired lung function had a decreased ability to respond to a pulmonary insult from the mix.

INTRODUCTION

Emphysema that is induced by the intratracheal injection of elastase in hamsters provides an animal model of pulmonary dysfunction that has many of the features of human panlobular emphysema (Kaplan, 1973). The pulmonary lesions that have been described histologically involve disruption of the elastic elements with distortion and enlargement of alveoli (Hays *et al.*, 1975; Kuhn *et al.*, 1976). These alterations have been quantitated morphometrically and are characterized by an increase in the mean linear intercept and by a decrease in the internal surface area (Kaplan, 1973; Snider and Korthy, 1978). Physiological measurements of pulmonary function in these impaired animals indicate that lung volumes and lung compliance increase while flow-volume events are altered in a manner similar to those in humans with emphysema (Snider *et al.*, 1977). The present study used this model to determine the effects of air pollutants on animals with experimentally induced lung disease. Animals with preexisting lung disease may

[1] This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

[2] This work was presented in part at the May 1981 Meeting of the American Thoracic Society, Detroit, Mich.

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be more sensitive than normal animals to lung damage from a variety of environmental and chemical exposures.

MATERIALS AND METHODS

Experimental animals. Sixty male golden hamsters (Charles River Breeding Labs, Wilmington, Mass.) weighing 104.8 ± 7.5 (SD) g were initially divided into two treatment groups. Half of the animals (EMP) received 6 units (U) of chromatographically purified porcine pancreatic elastase (Elastin Products Company, St. Louis, Mo., 117.7 U/mg), in 0.2 ml Tris buffer by a single intratracheal instillation. This dose of elastase produces a mild degree of emphysema which can be detected by tests of pulmonary function (Raub *et al.*, 1982). The remaining animals (CNT) received a sham injection of the Tris buffer alone. The hamsters were returned to the animal facility for a four week recovery period. Food and water were provided *ad libitum*.

Exposure. Two of the hamsters in the EMP group died shortly after treatment. The remaining animals were divided into four groups (Table 1). All animals were placed in individual stainless-steel cages and exposed to filtered room air (AIR) or a complex mixture (MIX) of the reaction products from sulfur dioxide (SO_2), ozone (O_3), and trans-2-butene, 23 hr/day for 4 weeks. The exposures took place in Rochester-type chambers (Hinners *et al.*, 1968) which were opened for approximately 1 hr each day for routine animal care. Food and water were supplied *ad libitum* during the entire exposure period.

The MIX consisted of the by-products of a dark phase reaction of olefin, O_3, and SO_2 (Walter *et al.*, 1977; McNelis, 1974). Briefly, 2621 μg/m³ (1.0 ppm) SO_2 and 1962 μg/m³ (1.0 ppm) O_3 were mixed in a dark reaction chamber. When the SO_2 and O_3 concentrations had stabilized, 6883 μm/m³ (3.0 ppm) trans-2-butene

TABLE 1

BODY WEIGHT, LUNG WEIGHT, AND LUNG WATER FOR HAMSTERS EXPOSED TO A COMPLEX MIXTURE OF OLEFIN–OZONE–SULFUR DIOXIDE REACTION PRODUCTS[a]

Group[b]	*N*	BW[c] (g)	BW[d] (g)	LW_{wet} (g)	LW_{dry} (g)	Percentage lung H_2O
CNT–AIR	15	103.7 ± 8.7	141.5 ± 14.2	.702 ± .062	.195 ± .022	72.1 ± 1.2
CNT–MIX	15	103.9 ± 6.6	133.5 ± 8.8	.699 ± .047	.189 ± .018	72.9 ± 1.6
EMP–AIR	14	106.5 ± 9.2	142.4 ± 11.4	.752 ± .069	.209 ± .023	72.2 ± 1.4
EMP–MIX	14	105.2 ± 4.6	133.3 ± 12.4	.729 ± .061	.197 ± .030	73.0 ± 2.6

[a] All data reported as mean ± standard deviation.

[b] Control (CNT) and emphysematous (EMP) hamsters exposed to filtered air (AIR) and the complex mixture of olefin–O_3–SO_2 (MIX).

[c] Initial body weight before treatment.

[d] Final body weight 4 weeks after treatment.

was added. The O_3 concentration dropped immediately as the SO_2 was oxidized and aerosol particles began forming. The major by-product components are given in Table 2. The volume median diameter (VMD) was obtained by an electrical aerosol analyzer (Thermo Systems, Inc., St. Paul, Minn.). The O_3 concentration was monitored continuously by a chemiluminescence analyzer (Bendix, Model 8002, Lewisburg, W. Va.). The trans-2-butene and acetaldehyde were measured hourly by gas chromatography equipped with flame ionization detection (Varian, Model 1400, Walnut Creek, Calif.). Sulfur dioxide was monitored continuously by a total sulfur analyzer using a flame photometric detector (Bendix, Model 8300, Ronceverte, W. Va.). Sulfate and ammonium ions were measured from filter samples by ion chromatography (Dionex, Model 14, Sunnyvale, Calif.). The concentrations of SO_2, trans-2-butene, and acetaldehyde were confirmed by low- and high-resolution mass spectrometry analysis.

Pulmonary function. Pulmonary function was measured immediately following exposure. The hamsters were anesthetized with 50 mg/kg sodium pentobarbital (Nembutal) given intraperitoneally. A tracheostomy was performed and a 15-gauge Luer stub adapter (Becton–Dickinson, Rutherford, N.J.) was tied into the trachea. The animals were placed in a whole-body pressure plethysmograph for lung function measurements by methods previously reported (Raub *et al.*, 1982). Briefly, lung volume was obtained from pressure changes in the plethysmograph measured by a differential pressure transducer (Model MP45, Validyne, Northridge, Calif.) referenced to a chamber equal in volume to the heat sink and connected to atmospheric pressure through a high-impedance pathway. Airway pressure (P_{ao}) was measured by a differential pressure transducer (Model MP45, Validyne) referenced to the plethysmograph providing the pressure difference across the total respiratory system(P_{rs}). Following a short period of resting breathing, the airway was occluded at end expiration. End-expiratory volume (EEV) was calculated from recordings of airway and plethysmographic pressure using Boyle's Law. The animals were hyperventilated on a small animal respirator (Model 5255, Ealing Corp., Natick, Mass.) to increase minute ventilation and establish a uniform volume history. A pressure–volume curve was obtained during apnea by inflating and deflating the lungs with a syringe pump. Vital capacity (VC) was

TABLE 2
MAJOR REACTION COMPONENTS FROM A DARK REACTION MIXTURE OF *TRANS*-2-BUTENE, OZONE, AND SULFUR DIOXIDE

Reaction components	Concentration[a]	Method
O_3	0.08 ± 0.01 ppm	Chemiluminescence
SO_2c	0.76 ± 0.07 ppm	Total sulfur, FPD
T-2-Butene	2.05 ± 0.18 ppm	Gas chromatography, FID
CH_3CHO	1.71 ± 0.15 ppm	Gas chromatography, FID
SO_4	400 ± 50 μg/m³	Ion chromatography
NH_4	130 ± 20 μg/m³	Ion chromatography
VMD	0.13 ± 0.02 μm	Electrical aerosol analyzer

[a] All data reported as mean ± standard deviation.

measured between a P_{rs} of -15 and $+30$ cm H_2O. Respiratory system compliance (C_{rs}) was calculated by a nonlinear least-squares regression fit of the deflation pressure–volume curve.

Multiple-breath nitrogen washout curves were obtained by ventilating the animals at a fixed volume and frequency with 100% oxygen while recording airway nitrogen concentration (Model 505, Med Science Nitralyzer, St. Louis, Mo.). The slope of the log end-tidal nitrogen concentration vs breath number (N_2 SLOPE) was used to assess the distribution of ventilation. Single breath diffusing capacity for carbon monoxide (D_{Lco}) and residual volume (RV) were measured by gas dilution techniques previously described (Takezawa *et al.*, 1980).

Following completion of the pulmonary function measurements, the animals were exsanguinated and the lungs were removed. The heart and mediastinal tissue were dissected free and the lung weight was determined before and after air drying at 30 cm H_2O inflation pressure and 105°C. Wet to dry weight ratios were calculated as percentage lung water.

Morphology. A separate group of 15 hamsters was used to evaluate the histopathologic changes resulting from exposure to the MIX in order to confirm the presence of emphysema. Five CNT and five EMP hamsters were exposed to AIR and five EMP hamsters were exposed to the MIX. Each animal was anesthetized with sodium pentobarbital (Nembutal) and a 15-gauge Luer stub adapter (Becton–Dickinson) was inserted into the trachea. The abdomen was opened and both hemidiaphragms were punctured to collapse the lungs. A 2% cacodylate-buffered glutaraldehyde solution was instilled intratracheally at a pressure of 30 cm H_2O. After a minimum of 30 min fixation, the lungs were dissected free. Sagittal sections of the left lobe were processed and stained with hematoxylin and eosin for light microscopy.

Statistical analysis. A one-way analysis of covariance was used to examine the effects of the two factors of interest, elastase treatment, and pollutant exposure, on the weight-adjusted means of each pulmonary function measurement. The possible interaction of these two factors was also examined. When the F values were significant, appropriate multiple comparisons among the means were made using Dunnett's test (Dunnett, 1955). Significant differences were indicated at the 0.05 probability level.

RESULTS

Effects of Elastase Treatment

Due to variations in animal size, the physiological data were adjusted for body weight. All of the physiological measurements showed changes indicative of emphysema. Lung volumes (Fig. 1) and C_{rs} (Fig. 2) increased ($P < 0.05$), while the N_2 SLOPE and D_{Lco} (Fig. 3) decreased ($P < 0.05$). There were no significant differences in either lung or body weights between CNT hamsters and hamsters with EMP (Table 1).

Effects of Complex Mix Exposure

There were no significant changes in either wet or dry lung weights or percentage lung water (Table 1). Lung volume and compliance were also not signif-

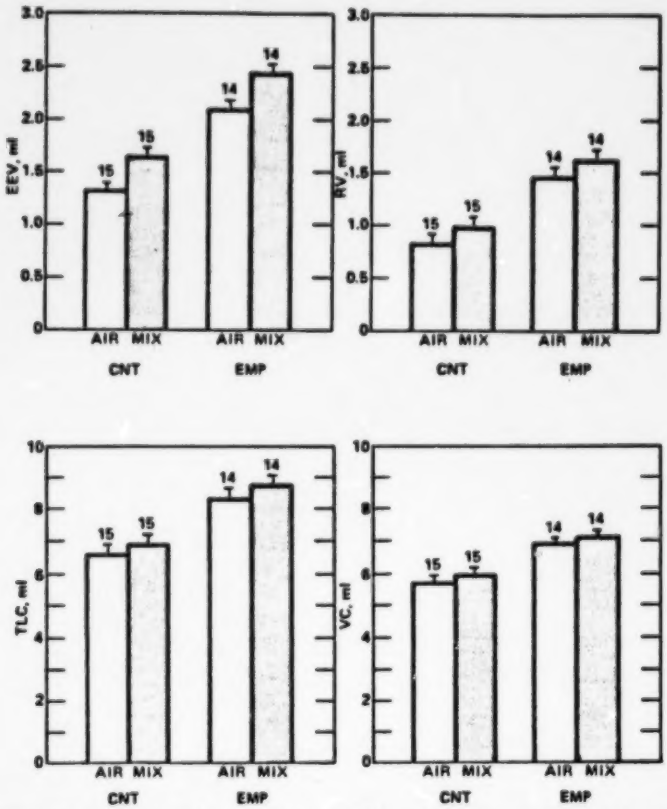

FIG. 1. Comparison of end-expiratory volume (EEV), residual volume (RV), vital capacity (VC), and total lung capacity (TLC) in normal hamsters (CNT) and hamsters with elastase-induced emphysema (EMP) following exposure to filtered air (AIR) and a complex mixture of olefin, ozone, and sulfur dioxide (MIX). The bars represent the standard error of the mean. There were significant differences ($P < 0.05$) between CNT and EMP hamsters for all lung volumes.

icantly altered by MIX exposure (Figs. 1 and 2). The effects of MIX exposure on N_2 SLOPE and D_{Lco} in CNT and EMP hamsters are shown in Fig. 3. The N_2 SLOPE decreased ($P < 0.05$) and the D_{Lco} increased ($P < 0.05$) in the CNT and EMP hamsters exposed to the MIX. While both the CNT and EMP hamsters showed comparable decreases in N_2 SLOPE, the D_{Lco} increased to a greater extent in CNT hamsters following MIX exposure than did the EMP hamsters. That is, there was a significant interaction ($P < 0.05$) between the effects of MIX exposure and EMP on the measurement of D_{Lco}.

Morphology

Histopathologic lesions were observed in all hamster lungs from the group with EMP and the group with EMP exposed to the MIX. Minimal to moderate degrees

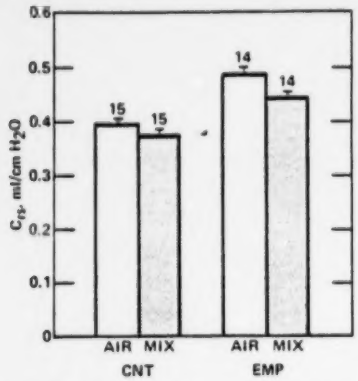

FIG. 2. Comparison of respiratory system compliance (C_{rs}) in normal hamsters (CNT) and hamsters with elastase-induced emphysema (EMP) following exposure to filtered air (AIR) and a complex mixture of olefin, ozone, and sulfur dioxide (MIX). The bars represent the standard error of the mean. There were significant differences ($P < 0.05$) between CNT and EMP hamsters.

of emphysema were present in the lungs of all these animals. Inflammatory lesions were characterized by septal thickening, intraalveolar accumulations of alveolar macrophages and polymorphonuclear cells, and perivascular accumulations of mononuclear and polymorphonuclear cells. The incidence and severity of these inflammatory changes were similar in both groups. However, an increased incidence of epithelial hyperplasia was found in the respiratory bronchioles, alveolar ducts, and proximal alveoli of the EMP hamsters exposed to the MIX.

DISCUSSION

Previous animal exposures to low levels of SO_2 or to mixtures with SO_2 showed no detrimental effect on pulmonary function (Alarie *et al.*, 1975; Antweiler and Brockhaus, 1976). Similarly, there have been no reports of physiological changes caused by O_3 levels as low as those found in the MIX. Recent investigations have tested the possibility of biological interaction among air pollutants. Amdur and co-workers (1978), using measurements of dynamic compliance and resistance in guinea pigs, were not able to demonstrate any synergism between O_3 and SO_2 at concentrations higher than those reported in the current study. The only published reports of synergism between O_3 and SO_2 have been from work on human subjects where both gases together showed a greater effect on ventilatory function than either gas alone (Hazucha and Bates, 1975; Bell *et al.*, 1977).

Earlier investigations with elastase-induced emphysema in hamsters have demonstrated a decrease in the surface area of the lungs (Kaplan *et al.*, 1973; Snider and Korthy, 1978). This is consistent with the decrease in diffusing capacity reported here and also by Goldstein and co-workers (1977). In addition, dose-dependent increases in lung volume and compliance and decreases in the N_2 SLOPE and single-breath D_{Lco} were observed over a wide range of intratracheally administered elastase doses in hamsters (Raub, 1982). As a result, 6 U of elastase/100 g body wt were used for this study because it produced a mild but measurable

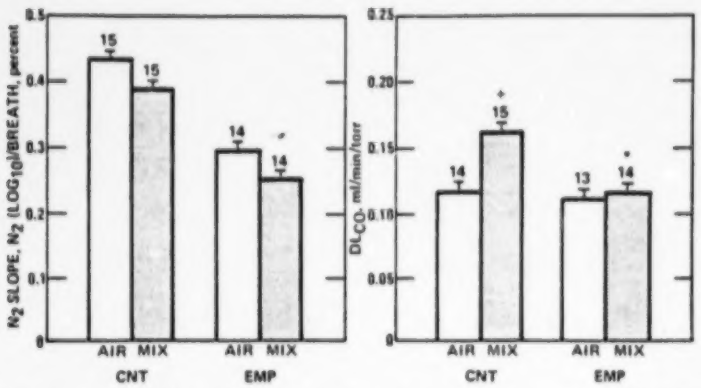

FIG. 3. Comparison of the slope of the nitrogen washout (N_2 SLOPE) and the single-breath diffusing capacity for carbon monoxide ($D_{L_{CO}}$) in normal hamsters (CNT) and hamsters with elastase-induced emphysema (EMP) following exposure to filtered air (AIR) and a complex mixture of olefin, ozone, and sulfur dioxide (MIX). The bars represent the standard error of the mean. There were significant differences ($P < 0.05$) in N_2 SLOPE between CNT and EMP hamsters as well as significant differences ($P < 0.05$) between AIR and MIX. Analysis of the interaction between MIX exposure and EMP shows: (a) MIX exposure decreases the N_2 SLOPE to the same extent in both CNT and EMP hamsters, and (b) the $D_{L_{CO}}$ is significantly increased in CNT hamsters exposed to the MIX, while hamsters with EMP are not able to increase $D_{L_{CO}}$ despite a more pronounced histopathologic lesion (see text for details). An asterisk (*) shows a significant difference ($P < 0.05$, Dunnett's test) between CNT and EMP hamsters. The symbol (+) shows a significant difference ($P < 0.05$, Dunnett's test) between hamsters exposed to AIR and MIX.

emphysema. This was particularly important in the current study because only mild changes were produced from exposure to the MIX. It would have been difficult to detect exposure-related changes in structure or function if these effects were obscured by severe emphysematous lesions produced experimentally by the injection of elastase.

A mild hypoxemia has been shown in hamsters with elastase-induced emphysema where differences in arterial PO_2 and in alveolar-arterial PO_2 and hematocrit were measured before and after moderate treadmill exercise (Lucey *et al.*, 1980). Even with increased metabolic loads, the impaired animals, like the controls, were still able to increase alveolar ventilation with exercise. This response was not observed in the current study since the emphysematous hamsters did not compensate for air pollutant exposure like the control animals. If a preexisting hypoxemia were present in the hamsters with emphysema, then any additional pathological lesions caused by complex mix exposure could have added to the impairment of gas exchange. Epithelial hyperplasia was found in the gas exchange regions of these animals. Part of the compensatory response to this damage may have been an increase in the EEV which was present in both the control and emphysematous hamsters but was not statistically significant. This could result in an enlargement of the diffusing area in the remaining gas exchange tissue counteracting the hypoxemic effects due to pathological changes in the lung parenchyma. However, due to a reduction in alveolar surface area, the animals with

emphysema simply do not have the reserve surface area which can be recruited to improve gas exchange.

Results of this study suggest an association between subchronic exposure to low levels of a complex pollutant mixture and measurable alterations in lung function. The distribution of ventilation and the D_{Lco} were significantly changed in both control and emphysematous hamsters following exposure to the complex mixture. Although the N_2 SLOPE decreased to the same extent in both groups, the effect on the distribution of ventilation may have had a greater influence in the emphysematous hamsters due to their reduced ventilatory efficiency. In addition, D_{Lco} was increased in normal hamsters following exposure to the mixture while measurements of D_{Lco} in the emphysematous hamsters were not significantly different from controls despite increases in lung volume. This increase in D_{Lco} has been observed in rats 6 months after receiving 50 mg coarse volcanic ash by intratracheal injection (unpublished observations), but the precise mechanism is unknown. Both control and emphysematous hamsters exposed to the mixture had inflammatory lesions which were identified by light microscopy. This pathological damage may have contributed to a generalized condition of hypoxemia. All of these changes suggest that the impaired animals were not able to compensate for a pulmonary insult as well as normal animals.

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